Application of the relationship between pharmacokinetics and pharmacodynamics in drug development and therapeutic equivalence: a PEARRL review

Authors

Ioannis Loisios-Konstantinidis[a], Rafael L. M. Paraiso[a], Nikoletta Fotaki[b], Mark McAllister[c], Rodrigo Cristofoletti[d], Jennifer Dressman[a]

Author Information

[a] Institute of Pharmaceutical Technology, Goethe University, Frankfurt am Main, Germany, [b] Department of Pharmacy and Pharmacology, Faculty of Science, University of Bath, Bath, UK, [c] Pfizer Drug Product Design, Sandwich, UK and [d] Brazilian Health Surveillance Agency (ANVISA), Division of Therapeutic Equivalence, Brasilia, Brazil

Correspondence

Jennifer Dressman, Biocenter, Institute of Pharmaceutical Technology, Johann Wolfgang Goethe University, Max-von-Laue-Str. 9, Frankfurt am Main 60438, Germany. Email: dressman@em.uni-frankfurt.de

Abstract

Objectives The objective of this review is to provide an overview of PK/PD models, focusing on drug-specific PK/PD models and highlighting their value-added in drug development and regulatory decision-making.

Key findings Many PK/PD models, with varying degrees of complexity and physiological understanding, have been developed to evaluate the safety and efficacy of drug products. In special populations (e.g. pediatrics), in cases where there is genetic polymorphism and in other instances where therapeutic outcomes are not well described solely by PK metrics, the implementation of PK/PD models is crucial to assure the desired clinical outcome. Since dissociation between the pharmacokinetic and pharmacodynamic profiles is often observed, it is proposed that physiologically-based pharmacokinetic (PBPK) and PK/PD models be given more weight by regulatory authorities when assessing the therapeutic equivalence of drug products.

Summary Modeling and simulation approaches already play an important role in drug development. While slowly moving away from "one-size fits all" PK methodologies to assess therapeutic outcomes, further work is required to increase confidence in PK/PD models in translatability and prediction of various clinical scenarios to encourage more widespread implementation in regulatory decision-making.

Keywords

Pharmacokinetics/ pharmacodynamics (PK/PD), modeling & simulation, drug development, regulatory science, bioequivalence, therapeutic equivalence

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1 Introduction

Over the last decades pharmacokinetic/pharmacodynamics (PK/PD) models have been evolving rapidly, starting with the pioneering work in the 1960s, then moving from empirical descriptions to models based on mechanistic and physiological approaches and still evolving today in the form of state-of-the-art mathematical models describing the progression of diseases as well as entire biological systems, under the umbrella of systems pharmacology and computational biology. [1],[2],[3],[4],[5],[6],[7]

At the beginning of the conjunction of pharmacokinetics with pharmacodynamics, empirical models which were based on the shape of the effect-concentration curve and assumed that the pharmacologic response is directly related to the drug plasma concentration were introduced. Soon it was recognized that this scenario is only valid when the equilibrium between the plasma and the site of action is instantaneous, when the free drug concentration and the distribution to all tissues is the same (or remains proportionally the same) and when the system is at steady-state. A variety of these so-called steady-state empirical direct effect models have been reported in the literature: linear, power, hyperbolic, sigmoid (E_{max} model), logarithmic and logistic. Even though these models have been applied in a number of situations,[1],[8],[9] they have two important limitations. First and most important, they are time-independent (also referred to as static models). Second, they lack a mechanistic and/or physiological understanding of the underlying pharmacokinetics and pharmacodynamics.[10] For these reasons, non-steady state, mechanistic and physiologically based modeling approaches were introduced and these are more widely used these days in drug development.

In parallel to the developments in modeling approaches, major regulatory authorities have been moving slowly but surely from “one-size fits all” concepts to a more case-by-case, scientifically justified approach, in which the application of modeling and simulation (M&S) is playing a valuable supporting role. Physiologically-based pharmacokinetic (PBPK) and PK/PD models have already been implemented in the assessment of drug-drug interactions (DDIs) and extrapolation of results from adults to pediatric

populations. [11],[12],[13],[14],[15],[16] In addition, generic dermatologic and inhalation products have been approved based on pharmacodynamic or clinical endpoint bioequivalence studies (BE).[17],[18]

Most recently, pharmacokinetic metrics providing information about delivery of the drug to the body and exposure (i.e. onset and duration of action),[19] such as partial areas under the concentration-time curve (pAUCs) have been recommended by the US-FDA for the evaluation of several complex oral products combining immediate (IR) with extended release (ER). [20],[21],[22] However, there are still many cases, especially for systematically acting drugs, where the value of modeling and simulation methods has not yet been widely recognized by the regulatory authorities. Such cases include the virtual bioequivalence of oral drug products, the justification for potential extension of BCS-based biowaivers to some BCS class II compounds and the reduction of the number of volunteers for bioequivalence studies of highly variable drugs (HVDs). In view of the fact that single point pharmacokinetic metrics (i.e. C_{max}, AUC) used to assess bioequivalence do not always comprise an appropriate surrogate for therapeutic equivalence (TE), which by definition is the ultimate goal of bioequivalence studies,[23] it would seem appropriate to implement modeling and simulation approaches to assure therapeutic outcomes in this arena too.

The aim of this review is to provide an overview of existing non-steady state PK/PD models, focusing on drug-specific case examples. These are intended to serve as examples of the importance of mechanistic PK/PD models in assuring desired therapeutic outcomes in clinical practice and to encourage wider implementation of PK/PD in support of regulatory decision-making.

2 coThe effect compartment model

2.1 Overview

In many cases, the site of action of a drug is kinetically distinct from plasma and the equilibration between the plasma and the effect site is often rather slow. In such cases, there will be a temporal delay between the drug plasma (C_p) and effect site concentrations (C_e) and the effect will be a function

of C_e rather than of C_p. Even though bioanalytical methods have improved greatly over the last decades, measuring the concentration at the effect site often remains a challenge, due to the lack of tissue accessibility.

In 1970, a hypothetical compartment serving as a link between the pharmacokinetic and pharmacodynamic models to address the equilibration kinetics was introduced by Segre et al.[2] and was applied for the first time by Forester et al.[24] to describe the time-course of effect of various cardiac glycosides.[25] This approach, using a so-called «effect compartment» or «biophase distribution» model (Fig. 1), was further elaborated and described mathematically by Holford and Sheiner [3],[26] as follows:

$$\frac{dA_e}{dt} = k_{1e} \cdot A_p - k_{e0} \cdot A_e \quad (1)$$

Where A_p and A_e are the amounts of drug in the plasma (main compartment) and in the effect compartment, respectively, and k_{1e}, k_{e0} are the first-order rate constants for distribution and elimination from the hypothetical compartment, respectively.

Assuming that the effect compartment receives a negligible amount of drug and that distribution to and clearance from the biophase compartment are equal, the model can be simplified and then coupled with a pharmacodynamic model, for example a sigmoid E_{max} model:

$$k_{1e} \cdot V_p = k_{e0} \cdot V_e \quad (2)$$

$$\frac{dC_e}{dt} = k_{e0} \cdot \left(C_p - C_e\right) \quad (3)$$

$$E(C_e(t)) = \frac{E_{max} \cdot C_e(t)^{\gamma}}{C_e(t)^{\gamma} + EC_{e50}^{\gamma}} \quad (4)$$

where C_p, V_p, C_e, V_e are the concentration and the volume in the central and effect compartment respectively; E_{max}, EC_{e50} and γ represent the maximum effect, the concentration in the effect site required to reach 50% of the maximum effect and the sigmoidicity factor, respectively. Alternatively,

the hypothetical compartment could be coupled with a peripheral compartment instead of the central compartment. However, it is not very common to use samples obtained at the effect site (e.g. using microdialysis) or any other peripheral compartment as a pharmacokinetic surrogate.

A hallmark of the effect compartment model is the hysteresis observed in the effect-concentration plot due to the time delay between pharmacokinetics and pharmacodynamics. In fact, this is a common attribute of non-steady-state pharmacokinetic/pharmacodynamic models.[27] Well-known examples of drugs exhibiting a biophase distribution delay related response include neuromuscular blocking agents such as d-tubocurarine (see section 2.2) and pancuronium,[28] the calcium channel blocker verapamil,[29] and the bronchodilator theophylline.[30] Further cases that have been reported in the literature include quinidine, disopyramide, opioids such as pethidine, morphine, fentanyl, diclofenac, organic nitrates, benzodiazepines and digoxin.[31],[32],[33],[34],[35],[36],[37],[38] In the following section, the models for tubocurare, pancuronium, ibuprofen and morphine are used to illustrate application of the effect compartment model.

2.2 Applications and case examples

2.2.1 d-tubocurarine and pancuronium

The assumption of a direct relationship between pharmacokinetics and drug response has been questioned for more than half a century, as illustrated by the case of d-tubocurarine.

Already in the early 1960s, the first attempts to simultaneously model pharmacokinetics and pharmacodynamics, based on the available plasma concentration and effect data for d-tubocurarine, were made. In 1964, Levy implemented a log-linear model to describe the time course of d-tubocurarine response, assuming one-compartment pharmacokinetics following intravenous bolus administration, based on the results of Ryan et al.[39] The log-linear model assumed that the effect of muscular relaxation is a linear function of the logarithm of the amount of d-tubocurarine present in the plasma,while elimination of the amount of d-tubocurarine in the body occurs exponentially with

time. In such cases, the pharmacologic activity declines linearly with time.[1] In 1972, an open three-compartment model for the pharmacological effect of d-tubocurarine was proposed by Gibaldi et al.[40] The amount of drug in the central compartment at the time of recovery from neuromuscular block was deemed by these authors to be dose-independent. This observation, combined with the very rapid onset of action of d-tubocurarine, led the authors to the conclusion that the site of action is located in the central compartment,[40] implying instantaneous equilibration between plasma concentration and response. However, the data on which this model was based had been collected during the terminal elimination phase, during which a pseudo-equilibrium between plasma and tissues concentration is reached and the distributional delay is minimized.

By contrast, Hull et al.[41] showed that after administration of pancuronium, a similar to d-tubocurarine neuromuscular blocking agent, a linear relationship between the logarithm of concentration and the response is a poor predictor of the early phase response, in which a hysteresis between the concentration in any compartment and twitch depression is observed. By adding a biophase compartment, expressed similarly to equation (3), and assuming that same degree of paralysis (i.e. during onset and offset of action) is associated with the same C_e, they were able to empirically relate the intensity of pharmacologic effect to the concentration at the site of action at every time point using a fixed effect pharmacodynamic model.[41] In the case of d-tubocurarine, the effect compartment model, as described mathematically by Holford and Sheiner,[3],[26] was successfully applied as well. Plasma concentration and effect data after intravenous administration were analyzed from healthy subjects and patients with renal failure. The model was able to fit data from both groups without statistically significant differences in the pharmacokinetic or pharmacodynamic parameters between the two groups.[42] Interestingly, the equilibration half-life (4 minutes) for pancuronium estimated in a more empirical way by Hull et al.[41] was very similar to the one for d-tubocurarine reported by Sheiner et al.[42] using an explicit pharmacokinetic/pharmacodynamic model.

In parallel, Stanski et al.[43] explored the influence of various anesthetic agents on the muscle-relaxing effect of d-tubocurarine. Halothane induced-anesthesia, in comparison to anesthesia with morphine

and nitrous oxide, prolonged the equilibration half-life. An open two-compartment pharmacokinetic model coupled with a hypothetical effect compartment was implemented to fit both plasma and muscle paralysis data. Interestingly, changes in pharmacodynamic (k_{e0}, $t_{1/2ke0}$, EC_{50}), but not in pharmacokinetic, parameters were observed for patients under halothane anesthesia. Furthermore, it was possible to distinguish between the effects of the agents on the EC_{50} for muscle paralysis showing that halothane sensitizes the neuromuscular junction to d-tubocurarine. Provided that the diffusion of tubocurarine into the extracellular fluid of the muscle and the receptor affinity is high, the rate limiting step for the onset of action is the rate of muscle perfusion, which is inversely proportional to the equilibration half-life ($t_{1/2ke0}$).[43] Although the onset and the magnitude of response is dependent on muscle blood flow, the recovery from neuromuscular blockage is perfusion-independent and solely related to the drug-receptor dissociation rate.[44] The significant increase in $t_{1/2ke0}$ under halothane-induced anesthesia is consistent with the decreased muscle blood flow, which would suggest a later onset of paralysis. However, halothane also decreases the EC_{50}, which compensates for the decrease in perfusion and results in a similar onset to that observed under morphine and nitrous oxide anesthesia.

In summary, the evaluation of the pharmacodynamics in concert with the pharmacodynamics of these two muscle relaxants enabled a more mechanistic description of their dose-response characteristics and a better understanding of the drug interaction with the anaesthetic. These early successes triggered further interest in combining pharmacokinetics with pharmacodynamics to achieve a more mechanistic description of the relationship between dose, dosing and clinical effects.

2.2.2 Ibuprofen: dental pain relief

Ibuprofen was selected as a model drug to investigate the clinical relevance of bioequivalence metrics to the therapeutic effect. An analysis of 25 bioequivalence studies of Ibuprofen immediate-release oral dosage forms over a dose range from 200-600 mg showed that 14 of the studies failed to prove bioequivalence in C_{max}, even though AUC fell within the bioequivalence limits.[45] The authors reported

213 that Ibuprofen, a weakly acidic BCS class II compound, is at higher risk to fail bioequivalence because
214 of C_{max} variations. However, in cases where the plasma concentration is related non-linearly and/or
215 indirectly to the drug effect[46],[3] , the C_{max} and t_{max} values may not be accurate metrics for the
216 therapeutic response. For example, if the C_{max} is higher than anticipated this will not necessarily
217 translate to toxic effects. Likewise, if the C_{max} is lower, this will not necessarily result in lack of
218 efficacy.[47]

219 Dissociation between pharmacokinetics and pharmacodynamics is common for NSAIDS. This may be
220 because of delayed distribution to the biophase or related to an indirect response mechanism, for
221 example when the pharmacodynamic endpoint is the inhibition of inflammation mediators.[48] Pain
222 relief and antipyresis after administration of ibuprofen formulations have been extensively modelled
223 in different populations. In this section, the main studies for pain relief after third molar extraction are
224 presented, while studies investigating the antipyretic effect are addressed in section 4.2.1.

225 Third molar extraction pain models describe the postoperative onset of inflammation, with maximum
226 pain intensity occurring in 12 hours or less. Relief from pain associated with tooth extraction exhibits
227 high reproducibility and a low placebo effect, features that are important for differentiation among
228 various doses and thus for the identification of dose-response curves.[49],[50],[51],[52] The most commonly
229 evaluated endpoints in dental pain models are the *pain intensity difference* (PID) and *sum of pain*
230 *intensity difference* (SPID), the *pain relief* (PAR) and *total pain relief* (TOTPAR), the *time to re-*
231 *medication* (REMD), the *time to first perceptible pain relief* (TFPR) and *time to first meaningful pain*
232 *relief* (TFMP).[53][54]

233 In a double-blind, randomized, single- and multi-dose study of 254 adult patients, who had undergone
234 third molar surgery, Hersh et al.[50] reported a positive dose-response relationship for sum pain
235 intensity (SPID), total pain relief (TOTPAR), time to re-medication (REMD) and overall pain relief, after
236 administration of 200 and 400 mg of ibuprofen as a single-dose. During the multi-dose phase, no
237 significant differences between the two dose levels were detected. The authors concluded that

patients could benefit from higher doses for pain treatment immediately after the extraction, but that lower doses would be satisfactory thereafter. These results suggest that the single-dose approach adopted for bioequivalence testing might be over-discriminating for the assessment of ibuprofen formulations with regard to the maintenance of dental pain relief. Indeed, McQuay et al.[55] observed no significant differences between 200 and 400 mg of ibuprofen in a double-blind, randomized, placebo-controlled, single-dose study comparing the analgesic effect of 200 and 400 mg of ibuprofen with placebo and with 200 mg ibuprofen plus 50, 100 or 200 mg caffeine in 161 adult patients after third molar removal. In a further study, a positive dose-response relationship of ibuprofen over the dose range 50-400 mg with regard to sum of pain intensity difference (SPID) and total pain relief (TOTPAR) was reported by Schou et al.[54] However, in terms of TOTPAR the doses of 200 and 400 mg did not differ significantly.

A meta-analysis of data from 13 trials with total of 994 patients reported an absolute increase of only 9% (from 59% to 68%) in the number of patients who achieved at least 50% pain relief, when the dose of ibuprofen was doubled from 200 to 400 mg, meaning that 10 patients would need to be treated with the higher dose for just one of them to benefit. [56] The analysis indicates that the dose-response relationship is rather flat in the dose range 200 to 400 mg with respect dental pain relief by ibuprofen.

Li et al.[53] applied a pharmacodynamic model to investigate the onset and offset of dental pain relief after administration of effervescent and standard tablets containing 400 mg ibuprofen. As an endpoint, a categorical pain relief score was applied and treated as a continuous variable, in agreement with Lemmens et al.[57] The observed distributional delay of the response to ibuprofen was addressed by the addition of an effect-compartment model and the overall effect as the sum of placebo and drug was described as following:

$$\frac{d(C_e[t])}{dt} = k_{e0} \cdot \{C_p[t] - C_e[t]\} \quad (5)$$

$$f_d(C_e) = \frac{E_{max} \cdot C_e^{\gamma}}{C_e^{\gamma} + EC_{50}^{\gamma}} \quad (6)$$

$$f_p[t] = P_{max} \cdot \left(1 - e^{-k_p \cdot t}\right) \quad (7)$$

$$PR(t) = f_p[t] + f_d(C_e) + \varepsilon \quad (8)$$

where C_p and C_e are the drug concentrations in plasma and in the effect-site compartment, respectively; k_{e0} and k_p are the first-order rate constants for the placebo effect and equilibration, respectively; E_{max} and P_{max} are the maximum ibuprofen and placebo effect, $f_d(C_e)$ and $f_p[t]$ are the pain relief by ibuprofen and placebo, respectively; γ and EC_{50} are the sigmoidicity factor and the drug plasma concentration to achieve 50% of E_{max}, respectively; $PR(t)$ represents the pain relief score at a given time t and ε stands for the normally distributed residual variability.

The model was able to describe the pain relief score data adequately and the effect was directly related to the effect-site concentration, which increased much faster for the effervescent than the standard tablets, with the peak effect site-concentration occurring one hour earlier than for the standard tablet (1.0 h versus 2.0 h). The sigmoidicity factor was estimated to be 2.0 ± 0.43, confirming the relatively flat dose-response curve of ibuprofen.

More recently, a PBPK/PD model for Ibuprofen was developed and validated by Cristofoletti and Dressman[58] with the SimCyp Simulator® version 12.2 (SimCyp Ltd.), fitting antipyretic and dental pain relief pharmacodynamic models to pharmacokinetic and pharmacodynamic data already published in the literature. The main goals of this study were a comprehensive evaluation of the clinical relevance of bioequivalence criteria for ibuprofen immediate-release oral dosage forms and a risk assessment of waiving *in vivo* bioequivalence studies of such products. To simulate the pharmacokinetic and pharmacodynamic profiles, virtual populations similar to those enrolled in the clinical studies by Walson et al.[59] and Li et al.[60] in terms of age and gender ratio were generated, such that virtual trials for the dental pain relief model included 100 adults per trial aging between 18-40 years and receiving tablets of 100, 200, 280 or 400 mg of Ibuprofen. One-at-a-time sensitivity analysis for the gastric solubility, gastric emptying time (GET), apparent permeability coefficient (P_{app}) and small intestine pH

was conducted and the effect of applying different dissolution rates in the simulations on the resulting pharmacokinetic and pharmacodynamic profiles was also investigated.[58] The authors found that the dose-response curve for dental pain relief is shallow and as a result relatively insensitive to changes in plasma concentrations within the range 12-23 mg/L (applying an EC_{50} of 10.2 mg/L). Comparing the pharmacodynamic response after the simulated administration of 280 versus 400 mg Ibuprofen tablets to adults undergoing third molar extraction, no significant differences in the response occurred. Interestingly, although (under the assumption that the 400 mg tablet is the reference product and the 280 mg tablet is the test product in a virtual bioequivalence scenario) the test product would not be bioequivalent to the reference product in terms of pharmacokinetics (Cmax ratio ($C_{max\text{-}T}$/ $C_{max\text{-}R}$) of 0.7), the 280 mg tablet would be still considered therapeutically equivalent to the 400 mg tablet for dental pain relief in adult patients.

Cristofoletti and Dressman combined *in vitro in vivo* extrapolation with PBPK/PD model to simulate the effect of different dissolution rates from products containing ibuprofen free acid (IBU-H) and salts (IBU salts) and to investigate whether these would a) reflect reported differences in pharmacokinetics as well as whether b) differences in pharmacokinetics would translate into difference in the ability of ibuprofen to relieve dental pain in adults.[61] The model was able to adequately predict the observed pharmacokinetic profiles. The pain relief model by Li et al.[60] was adopted to simulate ibuprofen response. As expected from the faster dissolution of the products containing salt forms of ibuprofen, the 90% confidence intervals (CI) for C_{max} did not meet the average bioequivalence (ABE) acceptance criteria. However, pain relief scores elicited by ibuprofen free acid and salts were identical. Interestingly, the simulated peak effect-site concentrations for both IBU-H and IBU salts 400 mg were found to be higher than the estimated EC_{80}≈20 mg/L, indicating that the extent of pain relief would be insensitive to pharmacokinetic changes at this dose level. Importantly, the duration over which the effect-site concentrations are maintained above EC_{80} should be also taken into account. The authors concluded that the bioequivalence criteria for C_{max} might be over-discriminatory and not clinically

relevant for assessing therapeutic equivalence of ibuprofen products in terms of overall dental pain relief.

As illustrated by the example of ibuprofen, therapeutic equivalence is not always captured appropriately by simple plasma concentration measurements due to the insensitivity of the pharmacodynamic response to the pharmacokinetics in the dose range typically applied. From this case example, it is evident that the interaction of the drug pharmacokinetics with the pharmacologic response should be taken into account to set clinically relevant specifications ("safe spaces") for drug products. Modeling and simulation techniques would be a powerful tool in this direction, facilitating a regulatory transition from the current "one size fits all" bioequivalence paradigm to a scenario based on the clinically-based, specific PK/PD characteristics of the drug product and thus able to provide a more accurate assessment of therapeutic equivalence.

2.2.3 Anti-nociceptive effect of morphine

For drugs, which exhibit high biological target affinity and/or reach their site of action by active transport mechanisms, distribution to the biophase may or may not impose a rate-limiting step. Over the past few years, several specific transporters that may influence the distribution of drugs to their site of action in the central nervous system (CNS) have been identified.[62],[63],[64],[65] However, the number of pharmacokinetic/pharmacodynamic (PK/PD) studies exploring the functional role of these transporters in the distribution to the effect site are few. One interesting example is the anti-nociceptive effect of morphine, for which mechanism-based models of the biophase distribution within the central nervous system were established using intracerebral micro-dialysis.

Letrent et al.[66] investigated the effect of GF120918, a potent and selective P-glycoprotein (P-gp) inhibitor, on the pharmacokinetics and pharmacodynamics of morphine in rats, which were randomized into GF120918 pretreated, vehicle and control groups. The concentrations of both morphine and its metabolite, morphine-3-glucoronide (M3G), in serum were quantified and the anti-nociception was expressed as the percentage of maximum possible response (% MPR). A two-

compartment pharmacokinetic model, together with an effect compartment coupled to a sigmoidal E_{max} model was employed to simultaneously fit the pharmacokinetic and pharmacodynamic data. Among the pharmacokinetic (AUC, Cl, MRT, V_{ss}) and pharmacodynamic (k_{e0}, EC_{50}, γ) parameters evaluated, only the equilibration rate constant (k_{e0}) and the %MPR were significantly altered by pre-treatment with GF120918, indicating a faster onset and more intense action, respectively (p=0.0023). The increased pharmacodynamic response could not be attributed to pharmacokinetic changes or to the elevated M3G concentrations. Since M3G does not possess any anti-nociceptive properties,[67],[68],[69] the authors suggested that the inhibition of P-gp by GF1920918 might diminish the efflux of morphine from brain capillary endothelial cells, leading to more rapid distribution and higher concentrations of morphine at its site of action. These data were supported by Xie et al.[70], who demonstrated, using trans-cortical micro-dialysis, that morphine concentrations in the brain were increased (1.7-fold) after administration to mdr-1a genetic deficient rats, whereas the metabolite M3G was unaffected.

Evaluation of the kinetics of biophase distribution within the central nervous system by intracerebral microdialysis, which has already been successfully applied to the characterization of the distributional behavior in several cases [71],[70],[72],[73], is a promising tool for the development of more sophisticated, mechanism-based models, enabling as yet unexplained aspects of the pharmacodynamics of the central nervous system acting drugs to be illuminated.

3 Modeling of irreversible mechanisms of action

3.1 Overview

In this section, we describe some examples of drugs that act in the human body through irreversible inhibition at the site of action. In general, pharmacodynamic (PD) effects are initiated by the interaction of drugs with targets such as receptors, enzymes, ion channels, cell membranes etc. Such

interactions may be reversible, with a balance between association and dissociation of the drug with the target, or irreversible when a drug bonds covalently to the target or the dissociation rate is extremely slow for the relevant time span. As a result of these interactions, a cascade of events is triggered, leading to the pharmacological effect, which can either stimulate (agonist) or inhibit (antagonist) a physiological process.[74],[75]

In many cases, drugs that irreversibly inhibit a physiological process are transformed, as a first step, into reactive metabolites, which then bind covalently to their target, resulting in its inactivation. In order for the pre-existing situation to be reestablished, it is necessary to resynthesize the target. In such cases, the duration of action is likely to be independent of the pharmacokinetic half-life of elimination of the drug and instead depends essentially on the *de novo* synthesis of the target. The irreversible inactivation of endogenous enzymes or receptors caused by drugs e.g. the antiplatelet effect of aspirin after binding cyclo-oxygenase-1,[76],[77] the 5 α-reductase inhibitors,[78],[79] and the proton pump inhibition by proton pump inhibitors (PPI),[80],[81],[82] are often described using such turnover models. Further examples are drugs that trigger apoptosis in human cells, bactericidal antibiotics,[83] reduction of viral load due to the treatment with antivirals,[84] cell death processes induced by anticancer drugs[85] and cytotoxic drugs which cause myelosupression.[86]

In general, the turnover models that have been presented in the literature are based on the following differential equation:[87]

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R - f(C) \cdot R \qquad R(0) = R_0 \quad (9)$$

where *R* denotes the response produced by the drug, R_0 is its initial response value, k_{in} is a zero-order rate constant for the response, k_{out} is a first-order elimination rate constant and the function of the drug concentration $f(C)$ can be interpreted as a bimolecular interaction of the drug or its active metabolite with the target. This is the general equation representing the turnover rate of the response, however, more complex scenarios are also possible, requiring more mechanistic models to be developed as will be discussed later.

Figure 2 depicts a turnover model that can be applied to the interaction between the drugs with receptors, enzymes or ion channels. In the case of interaction with endogenous enzymes, the k_{in} and k_{out} parameters represent apparent rates of response formation and dissipation respectively and *f(C)* represents the effect as a function of drug concentration.

3.2 Applications and case examples

3.2.1 Proton pump inhibitors

Proton pump inhibitors (PPIs) were chosen as the drug model for this topic since their inhibition of the proton pump (H^+, K^+-ATPase) enzyme present in the parietal cells of the stomach is irreversible. To understand the mechanism of inhibition by the PPIs, models describing the turnover of H^+, K^+-ATPase have been described.

The PPIs are, in and of themselves, inactive drugs that require an acid environment for their activation. These weakly basic substances reach the general circulation after absorption from the gastrointestinal tract and then become concentrated in the acid compartment of the parietal cells present in the gastric mucosa. Following their activation by conversion to the sulphonamide form in the acidic intracellular environment of the parietal cells, a covalent bond occurs between the activated PPI and cysteine residues present in H^+, K^+-ATPase. This enzyme is responsible for the final step in the secretory gastric acid process.[81],[88],[89] As a consequence of the binding, the enzyme is inactivated and this results in suppression of acid secretion into the gastric lumen.[90],[80] PPIs inhibit both basal and stimulated gastric acid secretion, regardless of the nature of stimulation of the parietal cells. In order for the acid secretion to be re-established, *de novo* synthesis of H^+, K^+-ATPase is necessary.[90],[91],[92]

Even though the elimination half-life of PPIs is only 1-2 hours, the pharmacodynamic half-life of the inhibitory effect on H^+, K^+-ATPase is about 48 hours, rendering a rapid elimination (PK) but long duration of response (PD) to members of this class.[92],[93],[94] By comparison, the pharmacodynamics of

415 drugs that reversibly bind to the proton pump to decrease acidic secretion in the stomach, such as
416 cimetidine and other H_2 receptor antagonists, can be described with a direct response PD model.[95]
417 To construct a mechanistic PK/PD model for PPIs, several factors have to be considered: the
418 accumulation of PPI in the parietal cell, the amount of active enzymes present in the canaliculus of
419 parietal cell, the rate of *de novo* synthesis of new proton pump enzymes, the metabolism and
420 inactivation of PPIs, the extent of covalent PPI binding to the proton pump in the parietal cell and the
421 stability of this binding.[96] Because of this complexity, several different models have been proposed to
422 describe the relationship between PK and PD for this class of drugs. There are empirical models that
423 simply consider the turnover of the proton pump and those that are more mechanistic, taking into
424 account the relevant physiology and PPI characteristics. In this section we will focus on PK/PD models
425 that have been used to describe the difference between the elimination half-life (PK) of PPIs and the
426 temporal inhibition of acid secretion (PD) that results from binding of the PPI with H^+, K^+-ATPase.
427 Katashima and co-workers[95] were the first to publish a mechanistic PK/PD model for PPIs. In the first
428 study, a model relating the unbound plasma concentration (C_f) of lanzoprazole and omeprazole to
429 the inhibitory effect on stomach acid secretion was developed. This model, illustrated in Figure 3,
430 utilizes the apparent turnover process of H^+, K^+-ATPase to describe the relationship between plasma
431 concentration and the inhibitory effect of the PPIs on gastric acid secretion.[97]
432
433 According to this PK/PD model, the inactive form of the PPI is present in the plasma, and only after
434 reaching the acid environment of the parietal cells is it transformed into the active form. This form
435 then reacts with active H^+, K^+-ATPase according to a second order reaction with the rate constant, K,
436 to establish a covalent bond between the activated PPI and H^+, K^+-ATPase, resulting in inactivation of
437 the enzyme.
438
439 The total amount of proton pump (E_t) remains at a constant level (k_s/k_1) because H^+, K^+-ATPase is
440 synthesized, on the one hand, at a rate described by the rate constant, K_s, but also eliminated, on the

other hand, at a rate described by the first order rate constant k_1. The inactive proton pump recovers at a rate described by the first order rate constant k_2. Under these circumstances, the apparent turnover rate constant, k, is represented by $k_1 + k_2$. The time courses of variation in the amount of active H^+, K^+-ATPase (E) and the inactive fraction (E_c) are expressed by the following equations:

$$\frac{dE}{dt} = -K \cdot C_f \cdot E - k \cdot E + k_2 \cdot E_c + K_s \quad (10)$$

$$\frac{dE_c}{dt} = K \cdot C_f \cdot E - (k_1 - k_2) \cdot E_c \quad (11)$$

An *in vivo* pharmacokinetic and pharmacodynamic study in rats was conducted over a dose range of 0.006 - 3 mg/kg (IV) with omeprazole and lanzoprazole. Using the data from intravenous administration in rats, the estimated half-life of the proton pump was 27 times longer than the elimination half-life for omeprazole and 66 times longer for lansoprazole. Using the PK/PD model described above, good agreement between predicted and observed data was achieved for both drugs.

After their success with the PK/PD model in describing the data from rats, Katashima and co-workers[81] extended the model to human studies with pantoprazole (PPZ), lansoprazole (LPZ) and omeprazole (OPZ). The PK/PD analysis of these PPIs in humans was conducted using data obtained after oral administration of OPZ (40mg), LPZ (30mg) and PPZ (40mg). Again, good agreement between the predicted and observed values for the parameters was achieved. The estimated half-life of elimination for omeprazole was 0.854 h, for lansoprazole 1.66 h and for pantoprazole 1.52 h, while the apparent recovery half-life of the inhibitory effect on gastric acid secretion was 27.5 h for omeprazole, 12.9 h for lanzaprole and 49.9 h for pantaprazole. These results confirmed the divergence between plasma concentration (PK) and the inhibitory effect on gastric acid secretion (PD) of these there PPIs.

The mechanistic PK/PD model was extended by Puchalski and co-workers for lansoprazole.[82] Their model was set up to describe the intra-gastric pH time profile over a 24 hour period, enabling the circadian rhythm of acid secretion and food effects on intra-gastric pH to be taken into account. Using this model, the estimated value for lansoprazole half-life of elimination was 3.2h, somewhat longer

than in the Katashima model (1.66 h), while in the clinical study the pH had not returned to the baseline level after 24h. As this proposed model took into account several factors that can interfere in the PPI absorption and activation, it should be particularly useful in the design of clinical studies, the prediction of the optimal dosing regimen and the investigation of PPI effects in different patient populations.[82]

The inhibitory effect of PPIs on gastric acid secretion has also been described by Abelo and co-workers[80] using a simpler, empirical turnover model type I, as introduced by Dayneka et al.[98] (see section 4.1.1). In the basic turnover model shown in Eq. 12 and applied to omeprazole in Figure 4, it is assumed that the drug inhibits or stimulates the production of an effect, which can be characterized by the zero order k_{in} turnover and the elimination first order k_{out} rate constants as appropriate. The rate of change of the response (R) provoked in the absence of the drug is described with the following equation:

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R \quad (12)$$

According to Eq. 12 the acid secretion (AS) is directly proportional to the concentration of the active proton pump enzyme (E). Equation 13 can be used to correct for the placebo effect on acid secretion:

$$R = \frac{AS(Drug,t)}{AS(Placebo,t)} = \frac{E(Drug,t)}{E(Placebo,t)} \quad (13)$$

Omeprazole irreversibly removes the enzyme from the system at a rate proportional to the amount of enzyme and the inhibitor concentration. Irreversible removal of the enzyme results in a decrease in the response according to equation 14:

$$\frac{dR}{dt} = k_{in} - \left(k_{out} + k_{ome} \cdot C_p\right) \cdot R \quad (14)$$

For a given concentration of omeprazole, the value for R at steady state (R_{ss}) will be:

$$R_{ss} = \frac{k_{in}}{k_{out} + k_{ome} \cdot C_{pSS}} \quad (15)$$

This relationship states that with increasing omeprazole concentration, R_{ss} approaches zero.

Data from studies in dogs were used to predict the PK and PD parameters for omeprazole for this species, leading to a prediction for the half-life of elimination of 1.3 h and for the effective half-life for inhibition of acid secretion ($t_{1/2\ Kout}$) of 51h. Using allometric scaling, the predicted half-life for humans was 1.5 h and the effective half-life for inhibition of acid secretion ($t_{1/2\ Kout}$) was 71.7 h. The discrepancy between predicted (71.7 h) and observed (48) $t_{1/2\ Kout}$ in humans was attributed to differences in basal acid secretion between dogs and humans. [99]

Ferron and co-workers [100] also used the basic turnover irreversible PK/PD approach, in this case to describe the inhibition of gastric acid secretion by pantoprazole in rats and humans. The model was able to adequately describe the time course of gastric acid secretion in rats at all doses studied. The next step it was to apply it to gastric secretion data obtained after single or multiple oral or intravenous administration of pantoprazole in humans. The estimated half-life for pantoprazole was 0.5 h in rats and 0.8 h in humans, in agreement with the observed data in both species.

Both the mechanistic and empirical models described in this section were able to predict the discrepancy between the half-life elimination (PK) of PPIs and the time-course of inhibition of acid secretion (PD). The models were also successful in describing further characteristics of PPIs, namely that the effect in acid secretion inhibition of PPIs is linked to the extent of exposure (AUC), and that the onset of action is governed by the maximum concentration (C_{max}). Thus, PK/PD modelling provides a powerful tool for analysing/predicting effects achieved with other dosing regimens. To circumvent the use of invasive methods in clinical studies for monitoring the gastric pH and inhibition of gastric acid secretion, it would be necessary to build PK/PD models that can also predict the extent of acid inhibition in terms of the pH value and the duration over which the pH is kept above a clinically relevant threshold value (usually pH 4) by the PPI.

In conclusion, modelling and simulation clearly shows why PPIs, despite having a short plasma half-life, are able to have a long duration of effect. Such models enable better decisions to be made about dosing intervals and also help to identify the time-frames over which drug/drug interactions with PPIs may persist.

3.2.2 Acetylsalicylic acid

Similarly to the PPIs, aspirin (ASA) has a long duration of action, even though it has a short elimination half-life ($t_{1/2}$ 18-30 min).[101],[102] ASA inhibits platelet-derived thromboxane (TXB2), with approximately 60% inhibition still observed four days after discontinuation of ASA.[101],[102] This pronounced dissociation between the elimination half-life (PK) and the time-frame of drug action (PD) occurs because ASA binds covalently to TXB2 causing irreversible inhibition of this enzyme. The TXB2 activity can only be re-established by synthesis of new platelets, which is a process that occurs over a period of approximately 10-14 days.[101] Because platelets are not nucleated, they are unable to synthesize new COX-1, and for this reason platelet function will only normalize after the platelets that have been acetylated by ASA are removed from the systemic circulation and replaced by new platelets derived from megakaryocytes.[103]

The first model describing cyclooxygenase activity in platelets and the blood vessel endothelium after oral administration of aspirin was developed by Yamamoto and co-workers.[77] These authors used irreversible inhibition, with renewal by enzymatic turnover, to explain the long duration of the antiplatelet effect of aspirin in humans. In this study thromboxane B_2 concentrations and the percentage of prostacyclin production in the blood vessels were used as biomarkers.[77]

It has been suggested that non-selective COX-1 inhibitors, e.g. ibuprofen, could limit the cardio-protective effect of aspirin.[104] For this reason Hong and co-workers[76] developed a PK/PD model that was based on the turnover of the COX-1 enzyme, in which the irreversible inhibition by aspirin and the reversible binding by ibuprofen were both incorporated. The rate changes of free

enzyme concentration available for aspirin binding (E) and the ibuprofen-enzyme complex (EI) were described by the following equations:

$$\frac{dE}{dt} = k_{in} - k_{out} \cdot E - K \cdot C_{asa} \cdot E - k_{on} \cdot C_{ibu} \cdot E + k_{off} \cdot EI \quad (16)$$

$$\frac{dEI}{dt} = k_{on} \cdot C_{ibu} \cdot E - k_{off} \cdot EI - k_{out} \cdot EI \quad (17)$$

where k_{in} is the zero-order production effect rate constant, k_{out} is the first order elimination rate constant, K is the second-order rate constant for the irreversible enzyme inactivation by aspirin, and k_{on} and k_{off} are the association and dissociation rate constants for binding of ibuprofen on the enzyme. C_{asa} and C_{ibu} represent the aspirin and ibuprofen concentrations in the plasma, assuming that both drugs follow a one compartment PK model with first order rate constants for absorption and elimination.

The mechanistic PK/PD model was able to reflect the anti-platelet effect of aspirin administered either alone or concomitantly with ibuprofen. As well as simulating the PK and PD time courses, significant inhibition of the antiplatelet effects of aspirin in the presence of a typical ibuprofen regimen was also demonstrated.

The most mechanistic PK/PD model describing the effects of aspirin on COX-1 activity to date was proposed by Giareta and co-workers.[105] This model uses a population of megakaryocytes (MK) and peripheral platelets present in the blood circulation to describe aspirin's antiplatelet activity, as shown in Figure 5.

For the construction of the PK/PD model for aspirin, the inactivation of COX-1 by low dose aspirin and the recovery of COX-1 after stopping treatment were taken into consideration. Other physiological processes, e.g. the description of the megacariopoiese process responsible for the maturation and generation of new platelets, were also accounted for. The basic characteristics of the megacariopoiese process are shown in Figure 5. The schematic description of the resulting PK/PD model is shown in Figure 6. It consists of three linear compartments to describe the PK behavior of aspirin and two non-

linear compartments to describe the mechanism of inactivation of COX-1 (PD) in MK cells and in the platelets generated from them. A full mathematical description of the model has been published by Giaretta and co-workers.[105]

The PK and PD parameters of the model were inferred from the literature and calibrated by measurements of TXB2, which represents the COX-1 activity in peripheral platelets, in 17 healthy subjects and 24 patients with essential thrombocythemia (ET).[105] The model was able to reproduce both the mean TXB2 inhibition time in healthy patients and the reduced inhibition of TXB2 seen in patients with ET. Thus, this mechanistic PK/PD model may helpful to customize aspirin regimens under conditions of altered megakaryopoiesis.

In addition to the dissociation between PK (short half-life of elimination) and PD (long response period) demonstrated by the models described above, the dose-response relationship for platelet inhibition by aspirin is flat. Feldman and co-workers[101] demonstrated that even with a 10-fold increase in dose of aspirin, only a two-fold increase in response (inhibition of TXB2) was observed. Since doses of 81 and 325 mg of ASA are not significantly different with regard to this clinical response, applying a low dose of aspirin to prevent platelet aggregation is justified.[101]

In summary, mechanistic models of the pharmacodynamic action of aspirin on platelets appear to be useful for customizing the prevention of thrombus formation and for designing clinical trials in special patient populations e.g. the elderly, pregnant women, children, obese patients, etc. Indeed, regulatory authorities are increasingly relying on and encouraging the use of modeling and simulation to forecast changes in PK and PD in rare diseases and in special populations of patients in whom it is challenging to perform clinical trials.

3.2.3 Exemestane

Exemestane, an irreversible aromatase type I (Ar type I) inhibitor for the treatment of advanced breast cancer of postmenopausal women, provides a further, interesting example of irreversible binding and biological target inactivation.

In an open, three-period, randomized, crossover study of twelve healthy post-menopausal women Valle et al. investigated the effects of formulation (suspension *versus* tablet) and administration of food (i.e. fasted *versus* fed) on the pharmacokinetics and pharmacodynamics of exemestane. As had already been demonstrated by previous clinical trials, oral administration of exemestane (25 mg/day) inactivates peripheral aromatase, leading to a 85-95% decrease in basal plasma estrone, estradiol and estrone sulphate (EIS) concentrations in post-menopausal women with advanced breast cancer. [106],[107],[108] First, population pharmacokinetic models, consisting of a mono- or bi- exponential absorption and three compartment distribution function, with empirical Bayesian estimates for each individual were developed. Absorption lag times were determined for both absorption models. An inhibitory (type I) indirect response pharmacodynamic model (see more details in section 4.1), in which synthesis and elimination of EIS (which is indirectly related to aromatase activity) are governed by zero- and first-order rate constants, respectively, was implemented to describe the dissociation between plasma concentrations and the observed effect:

$$\frac{dC_{EIS}}{dt} = k_s - k_o \cdot C_{EIS} \quad (18)$$

$$\frac{dC_{EIS}}{dt} = k_s \cdot \left(\frac{C^{\gamma}}{C^{\gamma} + IC_{50}^{\gamma}}\right) - k_o \cdot C_{EIS} \quad C_{EIS}(0) = C_{EIS\,0} \quad (19)$$

where C_{EIS} is the plasma concentration of estrone sulphate, k_s is the zero order rate constant for synthesis and k_o is the first-order rate constant for elimination, C^{γ} is the exemestane plasma concentration, IC_{50} represents the exemestane plasma concentration at which 50% of inhibition is achieved and γ is the Hill-coefficient. This semi-empirical, non-linear mixed-effect modeling approach fitted the data adequately.

A more mechanistic model, incorporating the irreversible aromatase inactivation by exemestane, was also applied. In this model the aromatase concentration, Ar, is assumed to be the system variable controlling the rate of synthesis of EIS. The production and elimination rate of aromatase is in turn governed by a zero-order (k_{se}) and first-order (k_{oe}) rate constant, respectively. The irreversible inhibition of aromatase by exemestane is characterized by an increase in the elimination of aromatase and represented by a second-order rate constant k_i. Assuming that the concentration of EIS precursor is constant and the concentration of aromatase is known, the model is fully identifiable. The rate of concentration changes of EIS and Ar are defined by the equations:

$$\frac{dC_{EIS}}{dt} = k_s \cdot Ar - k_o \cdot C_{EIS} \quad C_{EIS}(0) = C_{EIS\,0} \quad (20)$$

$$\frac{dAr}{dt} = k_{se} - k_{oe} \cdot Ar - k_i \cdot C_{EIS} \cdot Ar \quad Ar(0) = Ar_0 \quad (21)$$

where Ar_0 is the baseline concentration of aromatase.

The adoption of a more physiological relevant mechanism of action in the model was expected to provide better results. Nevertheless, the goodness of fit was not significantly improved over the type I indirect response model. Despite being semi-empirical, the type I indirect-response model was able to predict the drug effect in different scenarios (i.e. doses, dosage regimens), providing an external validation. In a sense, the initial, indirect response type I model could be considered as a “collapsed” form of the mechanism-based model, under the assumptions that Hill-coefficient is equal to one (γ=1) and that the aromatase dynamics equation is solved at equilibrium and then substituted in the EIS equation. These assumptions appear to be justified in the case of exemestane, since the pharmacodynamic parameters do not change significantly in the data range studied and a value of Hill-coefficient 1.75 (γ=1.75) has been reported. Hence, a relatively flat dose-response is implied.

An almost 4-fold increase in the absorption rate of exemestane when administered as a suspension as compared to a tablet was detected, while food intake decreased the absorption rate. Interestingly, these differences were mitigated in terms of pharmacodynamic response such that the maximum effect and time to maximum effect were not significantly different among treatment groups. The authors concluded that even large differences in pharmacokinetics arising from formulation or administration with food were not translated to a meaningful difference in pharmacodynamics.

The example of exemestane is interesting for two main reasons: a) it illustrates that a mechanism-based model of irreversible pharmacodynamics can be transformed, depending on data availability or fast equilibration, to a simplified, "collapsed" model, without influencing the outcome appreciably, and b) observed differences in absorption patterns and food effects are not always clinically relevant, especially when there is a long delay between plasma levels and the elicited drug response. Again, these findings support the consideration of pharmacodynamics as well as pharmacokinetics when determining whether two drug products or two dosing scenarios are therapeutically equivalent.

4 Indirect response and feedback control models

4.1 Overview

Most pharmacological targets are subject to homeostatic mechanisms, characterized by continuous degradation on the one hand and re-synthesis of one or more biomarkers (e.g. enzymes, antibodies, circulating proteins or inflammation factors) to compensate for elimination on the other hand, which balance each other to maintain a stable steady-state. This is often referred to as the turnover process. Some drugs elicit their action by perturbing the steady-state, resulting in a temporary or a more permanent change in the marker value. Such mechanisms of actions, which do not affect the response itself but rather influence the turnover process, are inherently indirect and the models describing their effect-time course are usually referred to as turnover or indirect response models. These models

typically exhibit a delay between the drug concentration-time and response-time profiles. The amplitude of the response and the extent of the time delay are dependent on the turnover rates (synthesis and degradation) of the pharmacological target as well as the magnitude of the effect.

4.1.1 "Basic" and "extended basic" indirect response models

Nagashima et al.[109] were the first to implement an indirect response model, which was used to explain the anticoagulant effect of warfarin on the activity of the prothrombin complex. In 1993, Dayneka et al.[110] introduced four basic mathematical models describing the indirect pharmacological processes, according to which the production and loss of the response, R, are governed by zero- and first-order rate constants, k_{in} and k_{out}, respectively. The drug can inhibit or stimulate the synthesis and/or the elimination process as follows:

Model I (inhibition of k_{in}):

$$\frac{dR}{dt} = k_{in} \cdot \left(1 - \frac{I_{max} \cdot C}{C + IC_{50}}\right) - k_{out} \cdot R, \;\; R(0) = R_0 \qquad (22)$$

Model II (inhibition of k_{out}):

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot \left(1 - \frac{I_{max} \cdot C}{C + IC_{50}}\right) \cdot R, \;\; R(0) = R_0 \qquad (23)$$

Model III (stimulation of k_{in}):

$$\frac{dR}{dt} = k_{in} \cdot \left(1 + \frac{E_{max} \cdot C}{C + EC_{50}}\right) - k_{out} \cdot R, \;\; R(0) = R_0 \qquad (24)$$

Model IV (stimulation of k_{out}):

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot \left(1 + \frac{E_{max} C}{C + EC_{50}}\right) \cdot R, \;\; R(0) = R_0 \qquad (25)$$

where k_{in}, k_{out} are the zero order production and first order elimination rate constants, C is the drug plasma concentration, and EC_{50} and IC_{50} represent the drug plasma concentrations achieving 50% of the maximum stimulating, E_{max}, and inhibitory, I_{max}, effects, respectively.

These four basic models, which are illustrated in Figure 7, have been applied extensively and some examples have been summarized by Jusko and Ko.[4] The inhibition of basophil trafficking by methylprednisolone and the furosemide-mediated inhibition of water reabsorption from the tubules and collecting duct were assessed by Model I and II, respectively, while the stimulation of the cyclic adenosine monophosphate (cAMP)-induced bronchodilation by the β-adrenergic receptor agonist terbutaline was described by Model III. In a further example, it was shown that the increase in cAMP by terbutaline activates the cellular membrane sodium-potassium pump, resulting in an increase of efflux of potassium ions from the plasma into cells, an effect that can be described with Model IV.

These basic turnover models can be modified and/or extended to account for more complex physiological processes such as time-dependent production ($k_{in}(t)$),[111] the rate of loss of cells according to their lifespan[112],[113],[114] and capacity limited processes such as nonlinear synthesis and degradation functions.[115] Further, many physiological processes such as secretion of hormones and gastric acid, gene expression, cardiac output and blood pressure are known to be subject to circadian rhythms, which might influence the pharmacokinetics and pharmacodynamics of various drugs.[116],[117],[118] Symmetric circadian rhythms have been described by trigonometric functions, such as the cosine model introduced by Lew et al.,[119] whereas asymmetric circadian rhythms have been modelled with the addition of exponential, dual cosine or harmonic functions.[120],[111] The detailed mathematical formalism around these functions has been summarized by Krzyzanski.[121]

4.1.2 Signal transduction and feedback control indirect response models

When a sequence of events takes place between receptor binding or activation and the observable effect, this is referred to as signal transduction and can involve signaling cascades, activation or inhibition of secondary messengers, gene up- or down-regulation and mRNA transcription to functional proteins. By definition, every transduction process has two inherent attributes: the transformation of the original signal and the introduction of a time-delay.[122],[123] Depending on the experimental time-scale, the time delay might or might not be discernable and in the latter case the response is described by a transduction model with no delay, for example in the operational model of agonism introduced by Black and Leff.[124] This model has been applied to describe the pharmacokinetic/pharmacodynamic relationships of A_1 adenosine, μ-opioid and 5-HT_{1A} receptor agonists.[125],[126],[127],[128],[129] However, in other cases the time delay produced by the transduction process is significant and the mathematical models need to be adjusted accordingly. The most common approach is the so-called transit compartment model (Fig. 8), which has been applied to the modeling of the genomic effects of corticosteroids, in this case known as the 5^{th} generation model for corticosteroids, as well as myelosuppression and hematologic toxicity in cancer chemotherapy.[130],[131],[132],[133]

Most physiological processes are subject to feedback control and belong to the so-called autoregulation systems. The pharmacokinetic/pharmacodynamic (PK/PD) models that do not address these auto-regulatory mechanisms fail to provide a complete insight of the drug-exposure relationship and it has been shown that this can lead to underestimation of the drug's potency.[123] The feedback control indirect response (FC IDR) models (see Figure 9) usually incorporate terms proportional to the error signal itself, the integral and the derivative of the error signal in linear and, less commonly, in nonlinear combinations. There are also FC IDR models which include an additional state, the "moderator" state, which feeds back to alter the synthesis or turnover of the response.[134] Numerous applications of PK/PD models incorporating feedback regulation mechanisms have been published in the literature.[132],[135],[136] The example of (S)-citalopram, a widely used selective serotonin receptor inhibitor (SSRI), is presented in detail in section 4.3.

4.2 Applications and case examples

4.2.1 Ibuprofen: antipyretic response

As mentioned in section 2.2.2, the antipyretic effect of ibuprofen resulting from the inhibition of prostaglandin synthesis has been investigated in numerous clinical studies and an indirect response model has been applied to fit the reported pharmacodynamic data. In a single-dose, placebo-controlled, double-blind and parallel-group trial by Walson et al.,[137] the safety, efficacy, tolerability and dose-effect relationships of ibuprofen products, formulated as a suspension at doses of 5 mg/kg and 10 mg/kg to treat febrile children, were compared to liquid formulations of acetaminophen. The patients (N=127) were split into groups according to their initial temperature and on whether antibiotics were being administered concurrently. A positive dose-response relationship between ibuprofen suspension 5 mg/kg and 10 mg/kg in the higher temperature (102.6-104°F), non-antibiotic group was demonstrated, whereas in the lower temperature group (101-102.5°F) both doses were equally effective. However, the authors pointed out that the plasma levels necessary for maximum effective antipyresis of ibuprofen (approximately 10 mg/L) are achievable at doses even less than 5 mg/kg, implying a ceiling effect in the antipyretic response at doses of 5 mg/kg or higher.

Similar results in 178 children were observed by Wilson et al.[138] In a single-dose, placebo-controlled study, during which age and initial temperature were considered as co-variates, both the 5 and 10 mg/kg doses were significantly superior to placebo, but not different from each other in terms of maximum reduction in temperature. However, it was concluded, based on the temperature at 6 hours after administration, the change of temperature from the baseline value and the percentage of efficacy, that the 10 mg/kg dose was more effective. The effect of the age and the initial temperature value on the magnitude of the pharmacological action was also emphasized.

In a double-blind, randomized, single-dose study of 5 and 10 mg/kg ibuprofen to treat febrile children (N=153) Brown et al.[139] noted a dissociation between t_{max} and time of maximum temperature decrease and found no correlation between the extent of temperature change and plasma levels at

765 $t_{R,max}$ or 6 hours post-administration. Further, there was no evidence that pretreatment with
766 antibiotics, race or gender influenced the antipyretic effect. By contrast, age and initial temperature
767 were shown to be co-variates. Interestingly, after compartmental pharmacokinetic analysis, only the
768 pharmacodynamic, but not the pharmacokinetic parameters related to absorption (C_{max}, t_{max}) and
769 elimination (k_{el}, $t_{1/2}$), were affected by the age of the child. In a subsequent paper, Brown et al. [140]
770 implemented an effect-compartment model coupled with a sigmoid E_{max} pharmacodynamic model to
771 describe the antipyretic effect of ibuprofen in children and further elaborated the model by adding a
772 linear and/or sinusoidal cyclic function for the decrease in temperature as co-variates to fit their own
773 as well as previously reported data [138]. Values of the estimated sigmoidicity factor (γ) were 3.97 ± 0.58
774 and 4.27 ± 0.63 for ibuprofen 5 mg/kg and 10 mg/kg, respectively, implying that the dose-response
775 relationship for antipyresis in children might be steeper than for dental pain relief in adults.

776 Troconiz et al.[47] reported a temporal disconnection between t_{max} after administration to febrile
777 children of 7 mg/kg ibuprofen as a suspension or as effervescent granules dosed at 200 or 400 mg (0.5
778 for the suspension and 1.9 hours for the effervescent granules) and time of maximum decrease in body
779 temperature (3 hours in both cases), suggesting that the formulation and its pharmacokinetic behavior
780 has little impact on the antipyretic effect of ibuprofen. The antipyretic response of non-steroidal anti-
781 inflammatory drugs (NSAIDs) has been attributed to their ability to inhibit the synthetic pathway of
782 prostaglandins, particularly of prostaglandin E_2 (PGE_2), via an indirect mechanism.[141] The following
783 equation was derived to describe the pharmacodynamics of antipyresis by this mechanism:

784 $$\frac{dT}{dt} = k_{syn} \cdot \left(1 - E_{\max} \cdot \frac{C^{\gamma}}{C^{\gamma} + EC_{50}^{\gamma}}\right) - k_{out} \cdot T \qquad (26)$$

785 where dT/dt represents the rate of body temperature change with time, k_{syn} and k_{out} are the zero-
786 order and first-order rate constants for synthesis and degradation of the inflammation mediator (i.e.
787 PGE_2), respectively, T is the body temperature, $E_{\max}$ is the maximum antipyretic effect, EC_{50} is the

788 drug plasma concentration (C) required to achieve half of the maximum effect and γ is the sigmoidicity
789 factor.

790 The proposed pharmacokinetic-pharmacodynamic model fitted the antipyretic profiles well. The
791 estimated EC_{50} and k_{out} parameters were in agreement with those previously reported by Garg and
792 Jusko (6.18 versus 10.2 mg/L for EC_{50} and 1.17 versus 0.89 h^{-1} for k_{out}), who had also applied an
793 indirect response model.[142] The sigmoidicity factor was calculated to be 2.71 ± 0.18, suggesting a
794 relatively flat dose-response curve. In contrast to previous studies, however, age and initial
795 temperature did not elicit covariate effects. [138],[143]

796 Based solely on the differences in C_{max} and t_{max} between the suspension and the effervescent granule
797 formulations, a delayed onset of drug action would be expected for the effervescent granules.
798 Nevertheless, the maximum antipyretic effect was similar and occurred at the same time for both
799 formulations. Importantly, an almost identical mean effect time course of 200 and 400mg of Ibuprofen
800 effervescent granules in febrile children was observed, implying that at least for this formulation there
801 was no significant clinical benefit with a dose increase (Fig. 10). Therefore, the authors concluded that
802 the formulation-dependent pharmacokinetic differences are mitigated by the response mechanism,
803 leading to similar pharmacodynamic responses for both formulations at both doses in febrile children.

804 Using a verified PBPK/PD model Cristofoletti and Dressman simulated the antipyretic response with
805 virtual trials of 2, 5, 7 or 10 mg/kg dosing of Ibuprofen suspension to 100 febrile children per trial in
806 the age range of 2-11 years.[58] In terms of maximum decrease in temperature from the baseline value,
807 the 5, 7 and 10 mg/kg doses were proven to be significantly superior to 2 mg/kg but not statistically
808 different from one another. A rather flat dose-response curve (with EC_{50}≈6.18 mg/L) was confirmed
809 for the antipyretic effect in children. Under the assumption that the 7 and 10 mg/kg dose represent
810 the test and reference products, respectively, the test product would be bioinequivalent to the
811 reference in terms of C_{max} and AUC ratios ($C_{max,T}/C_{max,R}$ and $AUC_{max,T}/AUC_{max,R}$ around 0.7), but still
812 therapeutically equivalent in children. This conclusion is supported by the data from Troconiz et al.[47],

whose clinical trial demonstrated superimposable antipyretic profiles between ibuprofen suspension 7 mg/kg and effervescent granules 400 mg (normalized by children mean body weight as 11.8 mg/kg) after administration to febrile children.

4.2.2 Rosuvastatin

Of the currently available 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors, rosuvastatin is one of the most effective at lowering the low density lipoprotein (LDL) cholesterol. Mevalonic acid synthesis, which takes place in the liver, is catalyzed by HMG-CoA reductase and is the first irreversible stage of the cholesterol biosynthetic pathway.[144],[145],[146]

A pharmacokinetic/pharmacodynamic model was developed to predict the response of rosuvastatin to different dosage regimens and identify differences in response between morning (at 07:00 a.m.) and evening (at 06:00 p.m.) administration. For this purpose, Aoyama et al.[147] used a two-compartment pharmacokinetic model with first order absorption and elimination from the central compartment, which was then linked to a modified inhibitory indirect response pharmacodynamic model describing the plasma concentrations of mevalonic acid (MVA). The model was further extended by incorporating a time-dependent periodic function in the zero-order synthesis rate constant of mevalonic acid to account for the circadian rhythm, as introduced by Krzyzanski et al.[148],[149] The model is presented in Figure 11 and described by the following equations:

$$\frac{dR}{dt} = k_{in} \cdot \left(1 - \frac{C_p^{\gamma}}{C_p^{\gamma} + IC_{p50}^{\gamma}}\right) - k_{out} \cdot R \quad (27)$$

where R is the response, k_{in} is the time-dependent zero order rate constant for the increase in plasma MVA concentration, k_{out} is the first order rate constant for the decrease in plasma MVA concentration, C_p represents the plasma concentration of rosuvastatin, IC_{p50} is the plasma concentration at which k_{in} is reduced 50% and γ is the sigmoidicity factor. The time-dependent k_{in} to account for the circadian rhythm is defined as follows

$$k_{in} = k_m + k_{amp} \cdot \cos(2 \cdot \pi(t - tz)/24) \quad (28)$$

where k_m and k_{amp} represent the mean MVA synthesis and its amplitude rate constants, respectively, and tz is the acrophase time, during which MVA is synthesized at the maximum rate. The following function to describe the circadian rhythm of k_m was proposed by Krzyzanski et al.[148]:

$$k_m = k_{out} \cdot IC - \frac{k_{amp} \cdot k_{out}^2}{k_{out}^2 + (2\pi/24)^2} \cdot [\cos\left(\frac{2 \cdot \pi \cdot (tz)}{24}\right) - \left(\frac{2 \cdot \pi}{24 \cdot k_{out}}\right) \cdot sin\left(\frac{2 \cdot \pi \cdot (tz)}{24}\right)] \quad (29)$$

where IC is the initial plasma MVA concentration measured at 6 a.m., set to 4.32 ng/ml.

Application of the time course of rosuvastatin and mevalonic acid plasma concentration to the model enabled an adequate prediction of the clinical data reported by Martin et al.[150] A higher reduction ratio of 7.7% in the area under the plasma MVA concentration–time curves over 24 hours at steady state ($AUEC_{0\text{-}24}$) was observed after administration in the evening. Furthermore, sensitivity analysis on the pharmacokinetic parameters showed that changes in the pharmacokinetics have a greater effect on the $AUEC_{0\text{-}24}$ reduction ratio after morning than after evening administration. This was attributed to the circadian rhythm, with the acrophase time estimated to be 15.5 hours. The authors concluded that evening administration of rosuvastatin might be useful in clinical practice.[147] The main limitation of the model is that it is based only on the mean plasma pharmacokinetic and pharmacodynamic data. Therefore, it does not address the concentration at the effect site, which is the liver and not the plasma, or the inter-subject variability. Most importantly, the use of only one mean PK/PD data set raises questions about the identifiability of the estimated parameters and caution should be exercised in drawing conclusions about the validity of this model.

Since the liver is the effect site for the statins, uptake into the liver is an important factor in their efficacy. Multiple transporters of the family of the organic anion transporting polypeptide (OATP) family are abundant in the liver, facilitating the active hepatic uptake of endogenous substances and xenobiotics, including statins, from sinusoidal blood.[151],[152],[153],[154],[155] Rosuvastatin is a substrate of the organic anion transporting polypeptide 1B1, 1B2, 1B3, 1A2 and the sodium-dependent

taurocholate co-transporting polypeptide.[151],[156] The expression of OATP1B1 on the sinusoidal membrane of human hepatocytes is encoded by the gene *SLCO1B1*, which is subjected to single-nucleotide polymorphisms (SNPs). As already demonstrated for paravastatin, pitavastatin and simvastatin, such polymorphisms are associated with reduced OATP1B1 *in vitro* activity and markedly increased plasma concentrations.[157],[158],[159],[160],[161] Pasanen et al.[158] investigated the effect of *SLCO1B1* polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin, after oral administration in 32 healthy volunteers, with the following genotypes: *SLCO1B1* c.521CC (n=4), *SLCO1B1* c.521CT (n=12), *SLCO1B1* c.521TT (wild type, n=16). Significant increases in the $AUC_{0\text{-}48\ h}$ and C_{max} (65% and 79%, respectively) in *SLCO1B1* c.521CC subjects compared to the reference genotype, *SLCO1B1* c.521TT, were observed. By contrast, increases in the $AUC_{0\text{-}48\ h}$ (144% increase), but not the C_{max}, were reported after administration of atorvastatin. This study implies that the reduced OATP1B1-mediated hepatic uptake of rosuvastatin due to *SLCO1B1* polymorphism results in an increased risk of a reduced cholesterol-lowering effect as well as adverse effects such as myopathy and/or rhabdomyolysis.

Based on the model of Aoyama et al.,[147] a full PBPK/PD model was built in the SimCyp Simulator® by Rose et al.[162] to investigate the impact of polymorphic hepatic uptake (OATP1A1, OATP1B4) and efflux transposers (BcRP, MRP2) on the disposition, pharmacologic and toxic effects of rosuvastatin. First, plasma concentrations were linked to the cholesterol-lowering effect of rosuvastatin, according to the plasma AUC of MVA. The simulations performed with the PBPK/PD model showed a large increase in the mean plasma AUC infinity (AUC_{∞}) of rosuvastatin by 63% and 111% for the *SLCO1B1* c.521CT and *SLCO1B1* c.521CC, respectively, compared to the wild type (*SLCO1B1* c.521TT). Similarly, a significant increase in MVA plasma AUC of 30% and 35% for the same genotypes was observed. However, the hepatic unbound intracellular water concentration (Cu_{IW}) of rosuvastatin, which was predicted by a permeability limited liver model, was considered to be a more relevant driver of its pharmacodynamic effect. Interestingly, only a slight decrease in Cu_{IW} based AUC_{∞} of 5.7% and 9.6%, with a parallel decrease in MVA plasma AUC of 3.1% and 5.8% were reported for the heterozygote and homozygote,

respectively. The latter findings are in agreement with a number of studies showing that OATP1B1 c.521T>C SNP has either no or only a slight effect on the cholesterol-lowering response to statins,[163],[164],[165] and that when plasma concentrations were used as the input, the results were misleading.

With regard to toxic effects, the effect of genetic polymorphism on rosuvastatin-mediated myopathy was investigated by prediction of muscle concentrations using a perfusion-limited model. A strong correlation between plasma concentrations and the risk of muscle-related adverse effects was observed. Thus, in contrast to the results for the cholesterol-lowering effect of rosuvastatin, the plasma concentration appears to be a good surrogate for the concentration at the muscle when assessing the risk of statin-induced muscle toxicity in individuals with polymorphic hepatic uptake transporter activity. This result was also in agreement with an already published study.[166]

High inter-individual variability among the different genotypes, limited availability of accurate *in vitro* data and/or published clinical studies at different dose levels as well as incomplete understanding of the impact of transporters on pharmacokinetics and/or pharmacodynamics, are some of the limitations which restrict the robustness of the models for rosuvastatin and their confidence in simulating different clinical scenarios. Despite these limitations, rosuvastatin serves as a useful case example to demonstrate the potential of linking PBPK with PD model to enhance physiological understanding and improve the ability to assess the impact of transporters on the pharmacologic and/or toxic response. Of particular importance was the finding that, in some instances, parameters other than the plasma concentration are appropriate indicators of the therapeutic and/or toxic effect. This example illustrates that implementation of (PB)PK/PD models (even on an exploratory basis) can provide valuable information during clinical drug development and significantly contribute to the clinical ramifications of genetic polymorphism and facilitate an optimal dosing regimen.

4.2.3 Escitalopram

912 Selective serotonin reuptake inhibitors (SSRIs), such as escitalopram, block the neuronal reuptake of
913 serotonin (5-HT), resulting in increased neurotransmitter concentration at the terminal and somato-
914 dendritic areas. However, the auto-receptors 5-HT_{1A} and 5-HT_{1B}, which regulate the 5-HT release from
915 neurons by negative feedback control, are also situated at the terminal and somato-dendritic neuronal
916 parts, respectively (Fig. 12).[167] Intracerebral microdialysis can be used to measure the extracellular
917 concentration of 5-HT and thus its concentration at the site of action.[168],[169]

918 Bundgaard et al.[170] developed an indirect response PK/PD model for escitalopram, including a
919 moderator state (tolerance model) to account for the auto-inhibitory feedback. For this purpose,
920 different doses of escitalopram were administered intravenously at a constant infusion rate over 60
921 minutes in four groups (vehicle, 2.5, 5 and 10 mg/kg) of six male Sprague-Dawley rats and the response
922 was expressed as the change in extracellular 5-HT concentration. A two-compartment
923 pharmacokinetic model with first order elimination from the main compartment was used to fit the
924 individual mean unbound plasma concentration-time profiles for each dose group and the predicted
925 profiles were used as the input to drive the pharmacodynamic model. A type II basic indirect response
926 model was implemented to describe the inhibition of 5-HT reuptake. In this model, the increase in the
927 response, R, over the baseline value R_0, feeds back to the moderator compartment and stimulates the
928 production of the moderator, M. As a simplifying approximation, the rates in and out of M are
929 described by a first-order rate constant k_{tol}. An increase in M induces a negative feedback on the
930 generation of the response and thus enables the baseline value to be reestablished. The model is
931 illustrated in Figure 13 and described by the following equations:

932 $$\frac{dR}{dt} = \frac{k_{in}}{M} - k_{out} \cdot R \cdot I(C_p) \quad (30)$$

933 $$\frac{dM}{dt} = k_{tol} \cdot R - k_{tol} \cdot M \quad (31)$$

934 $$I(C_p) = 1 - \frac{I_{max} \cdot C_p^n}{IC_{50}^n + C_p^n} \quad (32)$$

where R, M and C_p represent the response, the moderator and the escitalopram unbound plasma concentration respectively, I_{max}, IC_{50} and n are the maximum inhibitory effect, the potency and sigmoidicity factor respectively, and k_{in}, k_{out} and k_{tol} represent the turnover rate, fractional turnover rate and feedback rate constants, respectively (see Fig.13). By setting equations 30 and 31 equal to zero, the initial baseline conditions are obtained:

$$k_{in} = k_{out} \cdot R_0^2 \quad (33)$$

$$R_0 = M_0 = \sqrt{\frac{k_{in}}{k_{out}}} \quad (34)$$

The feedback control model fitted the response-time data well. Between unbound plasma concentration and 5-HT response, a distinct time-delay was observed for all doses, leading to a counter-clockwise hysteresis loop. The development of tolerance was confirmed by the fact that the terminal phases of the hysteresis loops were not superimposable as a function of dose: the higher dose groups exhibited a lower response at the same concentration. Based on one-way analysis of variance (ANOVA) and post hoc analysis, maximal increases in 5-HT extracellular levels reached 337%, 424% and 456% of the baseline and the levels remained elevated for 135, 175 and 235 minutes at the 2.5, 5 and 10 mg/kg doses, respectively. Despite the significant differences in plasma concentrations, the basal response value was recovered within 360 min following the administration of all tested doses. In fact, neither the duration nor the magnitude of the response increased when the dose was increased from 5 to 10 mg/kg. These findings are in agreement with previous studies in rats, in which increasing the dose of escitalopram exhibited a ceiling effect in the extracellular levels of 5-HT in the frontal cortex, as measured by microdialysis.[171],[172]

The results from this study established the high potency (IC_{50}= 4.4 µg/L) of escitalopram, with almost complete (I_{max}= 0.9) inhibition of reuptake. A fast neuronal 5-HT reuptake with a half-life of less than 5 minutes ($t_{1/2k_{out}}$) was reported, whereas the half-life for the development of tolerance, $t_{1/2k_{tol}}$ was estimated at 10 hours. The importance of incorporating a moderator state to account for the

physiological homeostatic autoregulation mechanisms was demonstrated by comparison of the pharmacodynamic parameters of this more mechanistic model with the conventional effect-compartment model. The effect-compartment model predicted higher EC_{50} values at increased doses, which was inconsistent with the physiological response. In addition, Zhang and D'Argenio[123] used the same data sets to compare the performance of the basic model II inhibitory model with and without the addition of proportional and proportional-plus-integral feedback gain. When the feedback was omitted, the drug's potency was underestimated, while the model with the proportional-plus-integral feedback gain performed the best (lowest Akaike information criterion value).

These findings not only highlight the usefulness of implementing feedback control mechanisms in pharmacodynamic models, but also the importance of assessing the PK/PD at multiple doses. It is evident that when the autoregulation of the pharmacodynamic response is not taken into account, the evaluation of *in vivo* potency can lead to an underestimation of drug's potency and application of unnecessarily high doses. Additionally, feedback control models may be useful for the comparison of the pharmacodynamic behavior among SSRIs, to improve understanding of their antidepressant effects and as a guide to set effective plasma concentrations in clinical practice.

5 Outlook and concluding remarks

This review describes the large variety of pharmacokinetic/pharmacodynamic modeling approaches available to predict dose-concentration-effect relationships and to simulate various clinical scenarios. Models incorporating a physiological understanding of the underlying mechanism(s) of action of the drug and progression of disease can serve as powerful tools for exploring and predicting clinical drug product performance. Provided such models are adequately validated, they can also be implemented with confidence to drive model-informed decisions during drug development as well as at the regulatory level.

An even more complete understanding of a drug's therapeutic value would be possible if dose-concentration-adverse reactions relationships were to be simultaneously established through

toxicokinetic/toxicodynamic models, so that not only efficacy, but also safety can be evaluated. This is important, since dose-response curves may differ significantly between the therapeutic and adverse effects in different patient populations as well as among different indications of the same drug.

A current limitation of mechanistic models is that their complexity often leads to issues of identifiability and reproducibility of parameters. The commercially available physiologically based pharmacokinetic models are often implemented with mostly (or only) literature data. In these models the number of parameters is often far greater than would be required for application of classical compartmental models and it may be difficult to acquire reliable values for some parameters. The advent of more sophisticated analytical techniques such as microdialysis will promote a better understanding of the time profile of drug concentration at the effect site. In the meantime, to ensure maximum quality and to facilitate the interpretation of PK/PD models, transparency in the parameter values applied in the model, as well as in the underlying assumptions and the derived equations, together with harmonization based on good coding practice (GCP), is essential.

Once there is enough confidence in the translatability, estimation and prediction of preclinical and clinical PK/PD and systems pharmacology models, a move towards linking them with biorelevant *in vitro* tools to guarantee therapeutic equivalence will be another key step forward in the drive to link the laboratory to the patient, which seems not only promising, but also imminent. Bridging the gap between *in vitro, in vivo* and *in silico* methods by applying the Quality by Design (QbD) and the Biopharmaceutics Risk Assessment Roadmap (BioRAM),[173],[174] will allow pharmaceutical scientists to correctly assess the relative impact of formulation, dose and dosing interval during development of new drugs.

For the formulation scientist, modeling and simulation used in this way will assist in the selection of the most appropriate dosage form and to set formulation targets, knowing to what extent the formulation can be expected to steer the *in vivo* performance of the drug product. For the clinician, the approach helps to identify the dosing strategy which optimizes the efficacy/safety ratio.

For the analyst, modeling and simulation can provide guidance in setting clinically relevant dissolution specifications, taking into account not only which formulation factors steer the drug plasma concentration (critical quality attributes) but also how any differences in these will translate in the clinical outcome. In this context, robust PK/PD modeling approaches will play an essential role in model-informed drug development.

Finally, from a regulatory decision-making point of view, a seamless description of the relationship between the pharmacokinetic and pharmacodynamic characteristics of a drug together with a knowledge of how, and to what extent, formulation and formulation performance can influence the PK and PD, provides an excellent, clinically relevant basis for an integrated approach to assessing applications for drug approval. Currently, pharmacodynamics considerations are taken into account in the approval of labeling of new drug products, for example, whether taking the drug before vs. after a meal will influence efficacy. There is also a thrust towards virtual bioequivalence, for example using PBPK modeling to determine whether a change in the dissolution characteristics will impact the plasma profile significantly. A logical further step would be to combine these two approaches to optimize the approval process. Foreseen is a scenario in which the release testing in the laboratory reflects the release in the target patient population(s), the data are combined with verified PBPK models tailored to the target population(s) and then translated with PK/PD modeling into a prediction of the clinical outcome. This scenario would not only provide sponsors as well as the regulatory authority with more flexibility in the approval procedure, without sacrificing efficacy or safety, but also be a way forward to move effectively towards a more personalized medicine concept.

6 Acknowledgements

This work was supported by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 674909 (PEARRL)

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